Remarks

Reconsideration of this patent application is respectfully requested in view of the following remarks. Claims 1-4, 8, and 15-17 have been amended to address formal issues, and are supported in the Examples of Applicants' specification. New Claim 18 is supported, for instance, in subject matter of original claim 2. No new matter has been added. Claims 1-18 are pending.

Claims 1-17 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection, and respectfully request reconsideration in view of the following comments.

Regarding claim 1, part e), Applicants respectfully submit the term "an agitated condition" is well known to one of skill in the art, and one of skill in the art would understand that an agitated condition means to move with an irregular and/or rapid action, which may include any means so as to disturb the culture. Accordingly, Applicants respectfully submit that claim 1 is definite.

Regarding claims 2 and 3, these claims have been amended to correct any use of improper Markush language. Additionally, claim 18 has been added as a result of the amendment of claim 2, and is supported in original claim 2. Accordingly, it is respectfully submitted that claims 2 and 3 are definite.

Regarding claim 4, corrections have been made, as suggested in the Office Action. It is respectfully submitted that claim 4 is definite.

Regarding claim 8, the range of light level recited in claim 8 is now within the scope of claim 1, in view of the above amendment of claim 1, supported, for instance, at page 9, lines 6-11. Additionally, it is respectfully submitted that the feature of "a profusely branched filamentous pigmented calli" obtained in each embedded block is a result of the calli being subcultured by growing thin slices of pigmented calli as embedded cultures in agar plates, as recited in the method of claim 8. Accordingly, it is respectfully submitted that claim 8 is definite.

Regarding claim 15, adding plant growth regulators including α-naphthalene acetic acid and 6-benzylaminopurine further enhances the formation of somatic embryos through somatic embryogenesis of pigmented callus, resulting in the facilitation of rapid growth and morphogenesis in micro-propagules (Example 4). It is respectfully submitted that claim 15 is definite.

Regarding claim 16, the matter with respect to the antecedent basis issue regarding "harvesting period" and the relative language of "can yield higher biomass" have been addressed. The cultivation period has been defined as "at least 60 days", and is supported for instance, at page 9, lines 26-29 of Applicants' specification. Accordingly, it is respectfully submitted that claim 16 is definite.

Regarding claim 17, this claim has been amended to address the parenthetical subject matter. Further, the control plant is defined as a parent plant. Accordingly, it is respectfully submitted claim 17 is definite.

In view of the above amendments and comments, it is respectfully submitted that claims 1-18 are definite. Favorable reconsideration and withdrawal of the rejection is respectfully requested.

Claims 1-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dawes et al. in view of Mairh et al. Applicants respectfully traverse this rejection, and respectfully request reconsideration in view of the following comments.

Claim 1 recites a tissue culture method for cultivation of marine algae including subculturing excised pigmented callus on fresh agar plates to obtain pigmented oval or spherical shaped micro-propragules (step c) and subculturing the pigmented callus to achieve enhanced somatic embryogenesis and micro-propagule formation in pigmented filamentous callus (step d). Further, claim 1 recites cultivating algal biomass on a large scale in the sea by growing the young plantlets in enclosed perforated polythene bags (step f).

The present invention provides development of fast growing strains of algae under *in vitro* conditions and development of the method of producing micro-propagules on a large scale through somatic embryogenesis of pigment callus of anatomically complex thallophytic red and brown algae (page 10, lines 8-16 and Examples). The plantlets obtained through germination of somatic embryos have several advantages, namely, they are faster in growth and they produce

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variability as compared to the parent material. The present invention undergoes the step of embryogenesis, which has led to a larger number of plantlets raised through tissue culture, as somatic embryogenesis is a rapid method for propagation.

Dawes et al. made attempts to develop suitable methods for maintenance and propagation of selected clones of different cultivated varieties of *Eucheuma* using tiny vegetative fragments. The main drawback of such propagation using micro-cuttings is that the progeny will possess only parental features and does not have a pronounced advantage over the parental populations in expression of desired traits (page 2, lines 9-17 of Applicants' specification). In fact, Dawes et al. focuses on establishment of an inexpensive *in vitro* culture facility near the farming areas for maintenance of seed stock of *Eucheuma* using mostly branches and micropropagules in liquid cultures, the micropropagules being vegetative cuttings of 1-5mm segments (page 248 and Fig. 3; page 253 and Table 2B). In achieving this end, Dawes et al. makes various possible approaches, for instance, selection of source material from various parts of thallus, development of methods for aseptic culture, optimization of liquid culture medium, culture condition and influence of various plant growth regulators. These optimized conditions for *Eucheuma* are for branch and vegetative cuttings of 1-5mm segments.

Dawes et al. does not teach or suggest the claimed invention as recited by claim 1. The cited reference discloses conditions for culturing fragments including branch and vegetative cuttings (1-5mm). Dawes et al. does not teach or suggest culturing for callus induction as required by claim 1. To the extent Dawes et al. discloses cloning of branch segments to produce micropropagules, which Applicants do not concede, the micropropagules are vegetative micro-cuttings of plants. However, the present invention, as in claim 1, defines spherical or oval shaped particles developed directly from subcultured pigment callus (Fig. 3). Dawes et al. does not disclose subculturing excised pigmented callus on fresh agar plates to obtain pigmented oval or spherical shaped micro-propragules. Further, the cited reference does not teach or suggest subculturing the pigmented callus to achieve enhanced somatic embryogenesis and micro-propagule formation in pigmented filamentous callus. For at least these reasons, Dawes does not disclose the present invention of claim 1, and would not arrive at advantages provided from the features of the claimed invention.

Mairh et al. is directed to demonstrating the possibility of bag cultivation of *Eucheuma striatum* on an experimental scale in field conditions. However, the main drawback is that this resulted in decrease in their daily growth rate as compared to those grown in open waters without polythene bags (page 3, lines 18-21 in Applicants' specification). A

Mairh et al. does not further the teachings of Dawes et al. in accomplishing the present invention. In fact, Mairh et al. teaches culture of *Kappaphycus striatum* in a laboratory, in a tank and in the field (tide pools in the intertidal zone) using apical, middle and basal fragments incised obliquely, transversely and longitudinally. Thus, Mairh et al. does not tissue culture raised plantlets and they have not used it in open sea conditions. Thus, the cited reference does not use tissue culture as required in claim 1 of the present invention. For at least these reasons Mairh et al. does not remedy the deficiencies of Dawes et al. Accordingly, Dawes et al. and Mairh et al., either alone or in combination, do not lead to the features of the claimed invention as required by claim 1. For at least these reasons, it is respectfully submitted that claim 1 is patentable over the references cited. B

Regarding claim 8, the cited references also do not teach or suggest the calli subcultured by growing thin slices of pigmented calli as embedded cultures in agar plates to obtain a profusely branched filamentous pigmented calli in each embedded block. Therefore it is respectfully submitted that claim 8 is patentable over the references cited. C

Claims 2-7 and 9-18 depend upon and further limit claim 1, and also are patentable over the references cited for at least the reasons with respect to claim 1. Favorable reconsideration and withdrawal of the rejection is respectfully requested.

With the above amendments and remarks, Applicants believe the claims pending in this patent application are in a condition for allowance. Favorable consideration is respectfully requested. If any further questions arise, the Examiner is invited to contact Applicant's representative at the number listed below.

Respectfully submitted,

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Version With Markings Showing Changes Made to Application Serial No. 09/656,561

1. (Amended) A tissue culture method for cultivation of marine algae, said method comprising the steps of:

a) establishing axenic viable material of an algae for tissue culture by sequential treatment of the algal material in sterile sea water supplemented with domestic liquid detergent, povidine iodine and finally incubating the treated material in Provasoli enriched seawater (PES) medium with a broad spectrum antibiotic mixture and a fungicide for about 24 to 96 hours followed by thorough cleaning with [sterlie] sterile sea water to remove any traces of antibiotics and fungicide and blotting with sterile filter paper to obtain axenic explants;

b) culturing the axenic explants on agar plates fortified with PES medium at a temperature ranging between 20-25°C in the presence of cool white fluorescent lights at about 20-50 μ mol photon m^{-2} s^{-1} irradiance and a 12 : 12 light and dark cycle for induction of callus;

c) excising the callus from the explant after a period of at least 40 days and subculturing the callus on fresh agar plates in the presence of cool white fluorescent lights with 40-60 μ mol photon m^{-2} s^{-1} irradiance and a 12 : 12 light and dark cycle to obtain differentiated densely pigmented oval or spherical shaped micro-propagules;

d) subculturing thin slices of the pigmented callus in agar plates in Provasoli Enriched Seawater (PES) medium containing plant growth regulators, for a period of about 20 to 40 days, in the presence of cool white fluorescent lights of [40] 20-60 μmol photon m^{-2} s^{-1} irradiance and a 12 : 12 light and dark cycle to achieve enhanced somatic embryogenesis and micro-propagule formation in pigmented filamentous callus;

e) transferring the filamentous calli with somatic embryos to liquid PES medium in an agitated condition for morphogenesis and development of young plantlets with multiple shoots from propagules; and

f) cultivating algal biomass on a large scale in the sea by growing the young plantlets in enclosed perforated polythene bags.

2. (Amended) A method as claimed in claim 1_2 wherein the material for tissue culture is a Rhodophytic marine algae selected from the group of [Rhodophytic and Phaeophytic marine

algae comprising the] genera of *Eucheuma*, *Gigartina*, and *Chondrus*[, *Laminaria*, *Undaria*, *Ecklonia*, *Eisenia*, *Macrocystis*, *Sargassum*, and *Turbinaria*].

3. (Amended) A method as claimed in claim 1_2 wherein the material for tissue culture is an algae selected from the group of [algae comprising] *Eucheuma striatum*, *Kappaphycus* [*alvarezzi*] *alvarezii*, *Eucheuma cottonii*, *Eucheuma denticulatum*, *Eucheuma spinosum*, *Eucheuma alvarezii*, *Eucheuma procrusteanum*, *Gigartina intermedia*, *Gigartina exasparata* and *Chondrus crispus*.

4. (Amended) A method as claimed in claim 1_2 wherein the axenic explants comprise 1 to 6 mm long cuttings with 3-4 mm diameter and are selected from the upper [ordistal] or distal parts of the algae.

8. (Amended) A method as claimed in claim 1_2 wherein the calli is subcultured by growing thin slices of pigmented calli as embedded cultures in agar plates containing 0.3-0.6% agar and made in provasoli enriched seawater medium at 20-25°C in the presence of cool white fluorescent light at about 20-50 μ mol photon m^{-2} s^{-1} irradiance with 12:12 light and dark cycle to obtain a profusely branched filamentous pigmented calli in each embedded block.

15. (Amended) A method as claimed in claim 1_2 wherein the process of formation of somatic embryos through somatic embryogenesis of pigmented callus is further enhanced by [addition] adding plant growth regulators [such as] including α-naphthalene acetic acid and 6-benzylaminopurine.

16. (Amended) A method as claimed in claim 1_2 wherein [the] a harvesting period [of] after at least 60 days can yield a higher biomass than that of a control of parent plants or wherein the biomass yield can be maintained constant and [the] a cultivation period reduced from at least 60 days.

17. (Amended) A method as claimed in claim 1, wherein a two fold increase in growth in fresh weight [(fresh weight)] is achieved over a control of parent plants, without change in carrageenan product yield [(carrageenan)] and gel strength, through micro-propagule[s] formation from pigmented calli.